REMARKS

I. Status of the claims and application

Claims 26-83, 85, and 93, 96-112, 117-124, 126, 135, 136, and 138 are pending. Claims 26-83 and 85 are withdrawn from consideration. Claims 1-25, 84, and 86-92 were previously canceled without prejudice or disclaimer. Claims 94, 95, 113-116, 125, 127-134, 137, and 139-143 are canceled here without prejudice or disclaimer. Applicants reserve the right to pursue any of the canceled subject matter in one or more continuing applications.

Claims 93, 96, 97, and 117 have been amended for reasons that follow. Since none introduces new matter, Applicants respectfully request entry of these amendments.

i. Claim 93

Claim 93 is amended to recite the depository accession number for the claimed "SC20" chromosomal fragment, namely "FERM BP-7583." Applicants submit herewith a copy of the clone information deposited at the International Patent Organism Depository on May 9, 2001. Applicants have amended the specification to reference the accession number for the SC20 deposit, namely Accession Number FERM BP-7583 deposited with the International Patent Organism Depository on May 9, 2001.

Subsection (iv) of claim 93 is amended to clarify that the recombinant chromosome comprises at least two fragments "from different human chromosomes." Further, each fragment comprises "an antibody gene locus."

The specification makes clear that one characteristic of the claimed recombinant chromosome is its constituency of different human chromosome fragments, *e.g.*, fragments obtained from chromosomes 2, 14, 21, and 22. "[T]he inventors succeeded in transferring chromosomes or fragments thereof derived from human normal fibroblast cells into mouse ES cells and obtaining clones which were capable of stable retention of the chromosomes or fragments" (page 14, lines 5-9). Indeed, a recombinant chromosome of the present invention may comprise "fragments of human chromosomes #14 and #22" (page 46, lines 5-6).

The specification also makes clear that the "foreign chromosome or fragment thereof may contain an antibody gene. The antibody gene may be one or more sets of antibody heavy-chain and light-chain genes (page 17, lines 22-25); and that the "antibody is preferably an antibody of a mammal, more preferably a human antibody" (page 20, lines 12-14).

Similarly, the specification makes clear that the "human antibody gene may be a human heavy-chain gene, a human light-chain κ gene, a human light-chain λ gene, or a combination thereof" (emphasis added; page 20, lines 22-24).

Accordingly, the amendment is fully supported by the present specification and Applicants respectfully request that it is entered and made of record.

To expedite prosecution, Applicants have deleted "had not been adjacently located" language from claim 93 (iv).

ii. Claims 96 and 97

Claims 96 and 97 are amended simply for grammatical reasons and to ensure there is correct antecedent basis with the "fragment" element of claim 93.

iii. Claim 117

Claim 117 also is amended to recite the "FERM BP-7583" accession number. Subsection (b) of claim 117 is amended to clarify that the "second chromosome fragment" comprises a "human antibody gene locus" as well as a recombinase recognition sequence. In this regard, Applicants clarify that the desired site of latter sequence is between the second chromosome fragment and "the SC20 human chromosome #14 fragment."

II. Chromosomes 2 and 22 are not generated spontaneously but are fragmented according to a repeatable and predictable telomere-truncation method

Claims 93-126 are rejected under 35 U.S.C. § 112, first paragraph. The Examiner maintains that "the fragments (SC20, W23 and 6-1 clone) are essential to the claimed invention and the specification has not provided a repeatable method." Office Action at page 3.

Contrary to the Examiner's point of view, fragments of chromosomes 2 and 22 are not spontaneously generated. Applicants take this opportunity to point to and summarize various Examples of the present application detailing the precise – and repeatable – methods by which fragments from chromosomes 2 and 22 were generated.

Furthermore, Applicants submit herewith a copy of the clone information deposited at the International Patent Organism Depository on May 9, 2001, denoted under accession number FERM BP-7583.

Basically, chromosome 2 and 22 are fragmented at previously-introduced telomere sequence at a desired site within each chromosome. Specifically, the entire human chromosome 22 was introduced into chicken DT40 cell, whereupon a human telomere sequence was inserted into its LIF gene locus. See Example 82 (page 270), Example 83 (page 274). The telomere-inserted sequence facilitates truncation of the chromosome at the site of the telomere sequence. Hence, chromosome 22 is cleaved at the specific telomere site. See also Kuroiwa *et al.*, "Efficient modification of a human chromosome by telomere-directed truncation...," *Nucleic Acid Research*, 26 (14) 3447-3448, 1998 (appended as Exhibit A), which reports that a "predicted truncation at the LIF locus on the chromosome 22 was done in all of the targeted clones" (emphasis added; page 3448, first column).

Likewise, the entire human chromosome 2 was introduced into chicken DT40 cell and a human telomere sequence was inserted at its CD8A gene locus and the precisely-defined chromosome 2 fragment subsequently obtained. See Example 95 at page 310 entitled "Site-directed cleavage of human chromosome #2."

After obtaining chromosome 2 and 22 fragments via this predictable, telomere-induced method, a loxP site was inserted into each chromosome in a site-specific manner, as well as into a human chromosome 14 fragment. To produce the described λHAC hybrid, *i.e.*, a cojoined chromosome 22/14 fragments, a Cre recombinase enzyme was used to induce a translocation between the two loxP sequences. See Examples 93 (page 306) and 94 (page 309).

The fragments of chromosomes 2 and 14 were similarly cojoined via Cre-loxP translocation to produce the κHAC hybrid. See Example 97 at page 314 and Example 98 at page 320. Neither fragment was generated spontaneously. The person skilled in the art can readily produce these fragments, pursuant to the telomere truncation method, and cojoin them in a repeated manner, as presently disclosed.

It is unnecessary and improper, therefore, for the claims to be "limited to the SC20 and W23 fragments and the 6-1 clone" as proposed by the Examiner at page 5 of the Office Action. Accordingly, Applicants respectfully request that this rejection be withdrawn.

III. The rejections under Section 112, first paragraph are moot

Claims 127-143 are rejected under 35 U.S.C. § 112, first paragraph for allegedly failing to comply with the written description requirement and the enablement requirement. Purely for the sake of expediting prosecution, Applicants have canceled claims 113-116, 127-134, 137, and 139-143, drawn to chromosome 21 fragments. Claims 135, 136, and 138 are not drawn to chromosome 21 fragments. Accordingly, the rejections are moot.

IV. Tomizuka teaches neither a recombinant chromosome comprising fragments from different human chromosomes nor a precisely-located recombinase site and, therefore, does not anticipate claim 93

Claim 93 is rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Tomizuka *et al.*, Nature Genetics, 16, pp. 133-143, 1997. The Examiner, having previously withdrawn the art on October 23, 2002, now reinstates that previous rejection.

Tomizuka does not teach a recombinant chromosome that is made up of different chromosome fragments as presently required in claim 93. The Examiner is heard to agree with

this distinction, since the Examiner states that "[T]here is no recitation or requirement in the claim that the two fragments be from different chromosomes." Office Action at page 14.

Contrary to the Examiner's contention at page 14, third paragraph, claim 93 does require that the recombinase recognition sequence be located between the chromosomal fragments: "wherein the recognition sequence for the site-directed recombinase enzyme is located between the chromosome fragments." Tomizuka does not teach such a sequential order.

Accordingly, for at least these reasons, Tomizuka does not teach each and every element of claim 93 and, therefore, does not anticipate claim 93. Applicants respectfully request, therefore, that this rejection be withdrawn.

V. Conclusion

In view of the above remarks and amendments, it is respectfully submitted that this application is in condition for allowance. The Examiner is invited to telephone the undersigned at the number listed below if the Examiner believes such would be helpful in advancing the application to issue.

Respectfully submitted,

Date April 29, 2005

By U.S. Reg. #55,459
for: Stephen A. Bent
Attorney for Applicant
Registration No. 29,768

FOLEY & LARDNER LLP
Washington Harbour
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5143
Telephone: (202) 672-5404
Facsimile: (202) 672-5399

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Nucleic Acids Research, 1998, Vol. 26, No. 14 3447–3448

Efficient modification of a human chromosome by telomere-directed truncation in high homologous recombination-proficient chicken DT40 cells

Yoshimi Kuroiwa, Tokuyuki Shinohara[1], Tomomi Notsu[1], Kazuma Tomizuka, Hitoshi Yoshida, Shun-ichi Takeda[2], Mitsuo Oshimura[1] and Isao Ishida*

Central Laboratories for Key Technology, Kirin Brewery Company Ltd, 1-13-5 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan, [1]Department of Molecular and Cell Genetics, School of Life Science, Faculty of Medicine, Tottori University and CREST (JST), Nishimachi 86, Yonago, Tottori 683-0826, Japan and [2]Department of Molecular Immunology, Faculty of Medicine, Kyoto University, Kyoto 606-01, Japan

Received April 21, 1998; Revised and Accepted May 29, 1998

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ABSTRACT

Truncation of human chromosomes at desired sites by homologous recombination techniques enables functional and structural analyses of human chromosomes and development of human artificial chromosomes. However, this targeted truncation has been inefficient. We describe here an efficient method for targeted truncation in the chicken DT40 cells with a high homologous recombination rate. The human chromosome 22 was transferred into DT40 cells, where human telomeric repeat (TTAGGG)n was targeted to the *LIF* locus on the chromosome. Molecular and cytogenetic analyses showed that the predicted truncation at the *LIF* locus occurred in all of the targeted clones.

In order to produce a diverse repertoire of complete human antibodies in animals, we have been making mice with entire immunoglobulin loci by microcell-mediated transfer of human chromosomes into embryonic stem (ES) cells (1). During the course of experiments, it is suggested that the size of human chromosomes might be important for their germline-transmission. Thus, we tried to obtain a truncated human chromosome 22 where *Ig* λ gene exists by target integration of human telomeric repeat (2) into the chromosome for its germline-transmission.

Although the targeted truncation of a human chromosome has been reported, the efficiency was <0.01%, mainly due to the inefficiency of target integration of telomeric sequences (8/12 000) in addition to that of telomere truncation (1/8) (3). The chicken pre-B cell line DT40 is known to be homologous recombination-proficient (4) and enables the efficient modification of human chromosomes by gene targeting (5,6). Thus, it is expected that the efficiency of targeted truncation of a human chromosome may be raised in DT40 cells.

First, we transferred the human chromosome 22 into DT40 cells from mouse A9 cells by MMCT method (1). PCR analysis using chromosome 22-specific primers and FISH analysis with human COT1 probe confirmed the successful transfer of the chromosome 22. Next, the clones 52-18 were electroporated at 550 V and 25 μF with the plasmid construct (Fig. 1). Out of 80 clones resistant to both G418 and puromycin, eight gave 6.3 kb of PCR products containing *Sal*I sites (Fig. 1), indicating that the human telomeric repeat was integrated into the *LIF* locus at 22q12 just distal to the *Ig* λ *(7)*, at about 10% efficiency. Furthermore, PCR analysis using chromosome 22-specific primers indicated that the truncation at the *LIF* locus might occur in clones 67, 68, 328 and 343 (Fig. 2a). By FISH analysis using a probe derived from the plasmid pGK*Puro* (8), all of the eight clones underwent targeted truncation at the *LIF* locus (Fig. 2b). In clones 64, 212, 222 and 305 however, cells (<10%) with the intact chromosome 22 were also observed (data not shown), which may contribute to the above PCR results. It is possible that the timing of telomere truncation may be varied between the cells, resulting in different karyotypes within a single clone.

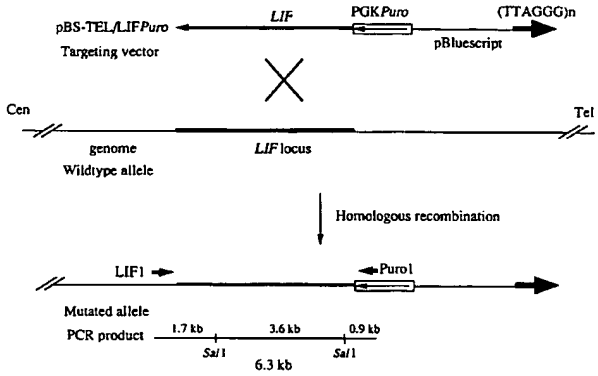

Figure 1. Strategy for targeted truncation at the *LIF* locus on the human chromosome 22 by the plasmid pBS-TEL/LIF*Puro*. Linearized pBS-TEL/LIF*Puro* is transfected by electroporation into DT40 cells. The identification of homologous recombinants was done by PCR using LIF1 primer (5′-ATGACTCTAAGGCAGGAACATCTGTACC-3′) and *Puro*1 primer (5′-GAGCTGCAAGAACTCTTCCTCACG-3′) indicated with bold arrows, followed by digestion with *Sal*I. In the targeted clones, 6.3 kb of PCR products should be amplified and digested with *Sal*I as indicated. The orientation of arrowheads in both *LIF* and PGK*Puro* represents that of their transcription.

*To whom correspondence should be addressed at present address: Pharmaceutical Research Laboratories, Kirin Brewery Company Ltd, 3 Miyahara-cho, Takasaki, Gunma 370-1295, Japan. Tel: +81 27 346 9922; Fax: +81 27 346 1971; Email: i-ishida@kirin.co.jp

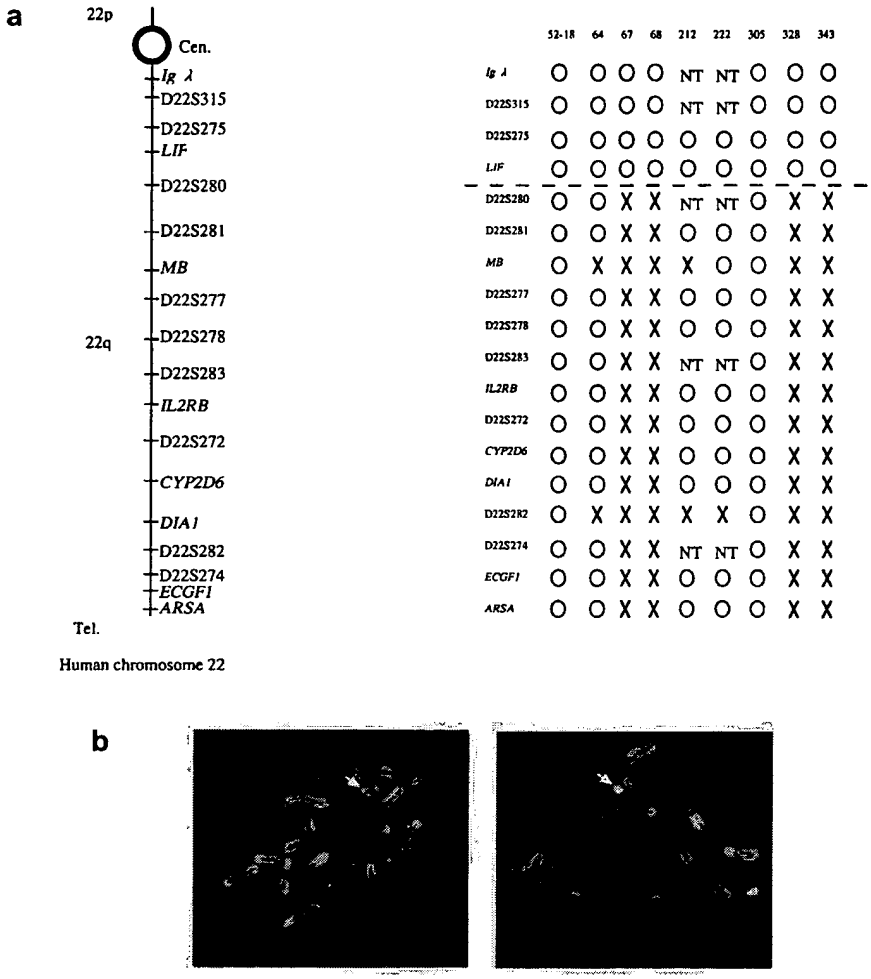

Figure 2. Molecular and cytogenetic analysis of the truncated human chromosome 22. (a) PCR analysis of the targeted clones using primers for the human chromosome 22-specific STS markers and genes. Their sequences are described elsewhere (1), except for both CYP2D6 (5′-CTGCGTGTGTAATCGTGTCC-3′ and 5′-TCTGCTGTGAGTGAACCTGC3′) and ECGF1 (5′-AGGAGGCACCTTGGATAAGC-3′ and 5′-TCACTCTGACCCACGATACAGC-3′). The left panel represents the approximate physical order of STS markers and genes tested, against centromere (7). In the right panel, symbols are as follows: O, presence; ×, absence. (b) FISH analysis of the targeted clones with both rhodamine-labelled human COT1 probe (red) and FITC-labelled plasmid pGK*Puro* probe (green). The left panel shows the partial metaphase of clone 52-18 with the intact human chromosome 22 (red) and the right panel shows the partial metaphase of clone 68, in which the site of hybridization of pGK*Puro* is telomeric (green).

In conclusion, the predicted truncation at the *LIF* locus on the chromosome 22 was done in all of the targeted clones (8/8) in DT40 cells. Furthermore, we also observed a high efficiency of targeted truncation in the human chromosome 3 as well (M.Oshimura, manuscript in preparation).The present finding is the first indication that DT40 cells are suitable hosts for telomere-directed truncation of human chromosomes, in addition to simple gene targeting and suggests that this technology is useful for detailed gene mapping by functional assays.

ACKNOWLEDGEMENTS

We thank Drs M.Shimizu and T.Yawata, Tottori University and Dr M.Koi, NIEHS for technical advice.

REFERENCES

1 Tomizuka,K., Yoshida,H., Uejima,H., Kugoh,H., Sato,K., Ohguma,A., Hayasaka,M., Hanaoka,K., Oshimura,M. and Ishida,I. (1997) *Nature Genet.*, **16**, 133–143.
2 Harrington,J.J., Bokkelen,G.V., Mays,R.W., Gustashow,K. and Willard,H.F. (1997) *Nature Genet.*, **15**, 345–355.
3 Itzhaki,J.E., Barnette,M.A., MacCarthy,A.B., Buckle,V.J., Brown,R.W.A. and Porter,A.C.G. (1992) *Nature Genet.*, **2**, 283–287.
4 Buerstedde,J.M. and Takeda,S. (1991) *Cell*, **67**, 179–188.
5 Dieken,E.S., Epner,E.M., Fiering,S., Fournier,R.E.K. and Groudine,M. (1996) *Nature Genet.*, **12**, 174–182.
6 Koi,M., Lamb,P.W., Filatov,L., Feinberg,A.P. and Barrett,J.C. (1997) *Cytogenet. Cell Genet.*, **76**, 72–76.
7 Collins,J.E., Cole,C.G., Smink,L.J., Garrett,C.L., Leversha,M.A., Soderlund,C.A., Maslen,G.L., Everett,L.A., Rice,K.M., Coffey,A.J. *et al.* (1995) *Nature*, **377** suppl., 367–379.
8 Watanabe,S., Kai,N., Yasuda,M., Kohmura,N., Sanbo,M., Mishina,M. and Yagi,T. (1995) *Biochem. Biophys. Res. Commun.*, **213**, 130–137.

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